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CONTRACTILE ACTION OF GALANIN ANALOGUES ON RAT ISOLATED GASTRIC FUNDUS STRIPS IS MODIFIED BY TACHYPHYLAXIS TO SUBSTANCE P

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This study was undertaken to characterize the interaction of porcine galanin (Gal) and some of its analogues with their receptors on rat gastric fundus muscle strips.

Gal, galantide (M15) and Gal(1–14)-[Abu^8]SCY-I evoked concentration-dependent contractions of gastric smooth muscle strips. Reproducible effects were observed in concentrations of 1–300, 3–1000 and 100–3000 nM, respectively. Specific EC_{50} for the contractile effect equalled 13, 70 and 187 nM.

Hill's coefficient for Gal is 1.03 indicating an interaction of one Gal molecule with one receptor, fulfilling the criteria of classical receptor theory. For M15 and Gal(1–14)-[Abu^8]SCY-I Hill's coefficients are different from 1, namely 0.73 and 1.56, pointing out that the principle of interaction of one drug molecule with one receptor may not apply. The contraction induced by 300 nM of Gal was not significantly modified by tachyphylaxis to substance P (SP). On the contrary the introduction of tachyphylaxis to SP decreased the contractile effects of M15 and Gal(1–14)-[Abu^8]SCY-I by about 57.7±3% and 39.6±5%, respectively. The findings suggest that contractile actions of M15 and Gal(1–14)-[Abu^8]SCY-I are probably not only due to their agonist activities at Gal receptors but may result from a subsequent stimulation of receptors for SP or release of endogenous SP.

KEY WORDS: galanin, smooth muscle, tachyphylaxis, substance P, rats.

INTRODUCTION

Gal, a 29 amino-acid, peptide was isolated from porcine intestine extracts by Tatemoto *et al.* [1]. Gal and its receptors are widely distributed in central and peripheral nervous systems, urogenital and gastrointestinal tracts of several mammalian species, including man [2–4].

In accordance with its widespread distribution, Gal is able to exert a plethora of different biological actions, including regulation of gastrointestinal motility [5], modulation of the pituitary–hypothalamus axis [6–7] and inhibition of pancreatic endocrine secretion [8]. Gal is also hypothesized to play a role in the memory and severe cognitive disabilities found in Alzheimer's patients [9].

Application of Gal and some of its analogues *in vitro* has been shown to result in concentration-dependent contractions of isolated gastric smooth muscle strips [1, 11]. Enough evidence exists to reveal the presence of specific Gal binding sites in the intestinal and gastric smooth muscles [10, 11]. It is important to emphasize the sensitivity of different sets of receptors to the action of the Gal analogue M15.

M15 is a selective antagonist of Gal receptors localized in the central nervous system [12] and blocks the Gal mediated inhibition of glucose-induced insulin secretion from the pancreatic β-cells [13]. In contrast, M15 acts as a full agonist of Gal receptors in the gastrointestinal smooth muscles [14]. M15 is a chimeric molecule consisting of the *N*-terminal part of Gal and a fragment of the SP moiety, namely Gal (1–13)-SP(5–11).

Another example of a peptide contracting gastric fundus muscles is a Gal analogue–Gal(1–14)-[Abu^8] SCY-I, containing a particle of scyliorhinin-I, modified by a substitution of glycine with α-aminobutyric acid in the 8th position of the chain. Both SP and SCY-I belong to the family of tachykinins [15]. Contractile action of M15 and Gal(1–14)-[Abu^8]SCY-I [16] may be at least partially produced by stimulating SP receptors. A hypothesis was proposed: tachyphyl-

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axis of gastric smooth muscle strips to the action of SP decreases the contraction evoked by M15 and Gal(1–14)-[Abu^8]SCY-I without a change in Gal action.

MATERIALS AND METHODS

Animals and tissue preparation

Male albino Wistar rats (weighing 180–250 g) were killed by a blow on the head. The abdominal cavity was opened using a midline incision, the stomach excised, the fundus cut off, and longitudinal strips prepared according to Vane (1957). Strips were suspended in organ baths (volume 15 ml) containing Tyrode's solution at 37°C bubbled with O_2/CO_2 (95:5), and kept at a resting tension of 2.0 g. The composition of Tyrode's solution (pH 7.4) was (mM): NaCl, 136.9; KCl, 3.35; $CaCl_2$, 1.46; $MgCl_2$, 1.03; $NaHCO_3$, 11.9; NaH_2PO_4, 0.48; glucose, 5.0. Responses of the stomach strips to the tested agents were recorded isotonically with PIT 212 transducers (C.O.T.M., Białystok, Poland), connected to a TZ 4100 line recorder (Laboratorni Pristroje, Prague, Czech Republic). Tissues were allowed to equilibrate for 60 min before the start of the experiment. Buffer was changed every 5 minutes, except for the contact time of the tested agent with the tissue, lasting up to 20 minutes.

Concentration–response curves for Gal, M15 and Gal(1–14)-[Abu^8]SCY-I

Experiments were commenced when reproducible contractile responses to carbachol (10–30 nM) were obtained. No more than two concentration–response studies were performed on each strip: a control one for Gal and another one for the Gal analogue. Only one Gal analogue was applied to each strip. Gal or its analogues were added in increasing concentrations, directly into the organ bath by cumulative additions, until a maximum contractile response occurred (i.e. when a contractile response could not be further increased by a higher concentration of peptide).

Contact time of a given peptide with muscle strips which allowed a development of the maximum contraction ranged from 15 to 20 min. Three minutes after the maximum contraction was reached the tissue was washed out at a rate of 2.5 ml/s for 2–3 min until the length of the strip returned to the basal level. Then the strip was left to equilibrate for 20 min (buffer changed every 5 min). Viability and reproducible contractility of each strip was examined at the end of each experimental session by a submaximal contractile response to carbachol, at the same concentration as at the start.

Tachyphylaxis to SP

Experiments were conducted in the same set as described above. Concentrations of Gal, M15 and Gal(1–14)-[Abu^8]SCY-I established to have caused maximal contractions were added to the organ bath. Three minutes after the maximum contraction was reached the tissue was washed. A fast, progressive diminution of the contractile response of the longitudinal gastric smooth muscle strips to the action of SP was obtained as described by Holzer-Petsche [18]. Briefly, a test concentration of SP (500 nM) was administered to the organ bath for 1 min. Then 5000 nM of SP was applied for 5 min. Four minutes after wash out, the test concentration was added again with the same contact time as before. One minute later the concentrations of Gal, M15 or Gal(1–14)-[Abu^8] SCY-I evoking a maximal contractile response in controls were added to the organ bath.

Drugs

Gal, M15 and Gal(1–14)-[Abu^8]SCY-I were manually synthesized by Rekowski, Halama and Mucha using the solid phase peptide synthesis procedure and later purified to homogeneity by high performance liquid chromatography using a System Gold Beckman chromatograph (for details see [19]). Gal(1–14)-[Abu^8]SCY-I is a chimeric peptide assembled from the *N*-terminal part of porcine Gal and SCY-I modified by a substitution of glycine in the 8th position of the chain with the α-aminobutyric acid molecule. SCY-I was originally isolated from the intestine of common dogfish [20, 21]. SP was purchased from RBI (Natick, MA, USA). All drugs were dissolved in Tyrode's solution. The detailed structure of peptides are presented in Table I.

Biostatistical analysis of acquired data

Results are expressed as a percentage of the maximum response induced by each peptide. The values of efficacies and potencies (EC_{50}) were expressed as means with respective confidence limits. Efficacy refers to the maximum response produced by the tested peptides and is expressed as a percentage in comparison to the maximum contractile effect produced by Gal.

Potencies (EC_{50}) were estimated using the computer program Pharmacological Calculation System-Pharm/PCS, version 4 based on the Manual of Pharmacologic Calculations with Computer Programs [22].

Values of respective efficacies and EC_{50} were compared using One Way Analysis of Variance (ANOVA) plus the Bonferroni post ANOVA test. To perform ANOVA and the test a GraphPAD INSTAT computer program, version 1.12a was used.

To determine if Gal or its analogue–receptor interaction follow a classical receptor theory, the Hill's coefficient was calculated using a program based on Biodata Handling with Microcomputers [23]. The contractile response produced by Gal, M15, Gal(1-14)-[Abu^8]SCY-I and the test concentration of SP were measured before (controls) and after tachyphylaxis to SP. Results were expressed as a percentage of respective controls. Values contained in the tables are

calculated as means of all experiments±SEM and compared using a two-tailed Wilcoxon signed rank test for pairs, with a GraphPAD INSTAT computer program, version 1.12a. A P value of less than 0.05 was taken to indicate a significant difference.

RESULTS

Effects of Gal and its analogues on rat gastric fundus strips

Gal, M15 and Gal(1–14)-[Abu^8]SCY-I evoked concentration-dependent contractions of rat gastric fundus strips. Reproducible effects for Gal were observed at concentrations of 1 nM. The maximal contraction was achieved at a concentration of 300 nM. The EC_{50} value of Gal equalled 13.7 nM and Hill's coefficient was 1.03 (Table II).

Detectable effects for M15 were observed at concentrations of 3 nM, with maximum contraction at 1000 nM. EC_{50} was 70.1 nM and Hill's coefficient 0.73 (Table II).

Effects of Gal(1–14)-[Abu^8]SCY-I were observed at a concentration of 100 nM, maximum contraction at a concentration of 3000 nM. Its EC_{50} equalled 187 nM and the Hill's coefficient was 1.56 (Table II).

Efficacy and EC_{50} of M15 and Gal(1–14)-[Abu^8]-SCY-I were significantly different from those of Gal (Table II).

Tachyphylaxis to SP

The regimen of inducing tachyphylaxis to SP led to a considerable reduction of the contractile effect of the test concentration of SP by 79.5±2.2% (P<0.001; n=30); a result very similar to the one obtained by Holzer-Petsche [18].

Effects of tachyphylaxis to SP on the contractile response of the rat gastric muscles to Gal, M15 and Gal(1–14)-[Abu^8]SCY-I

The contraction induced by 300 nM of Gal was not significantly modified by tachyphylaxis to SP (Table III). Tachyphylaxis to SP decreased the contractile

Table I
Structures of porcine galanin and its analogues

Peptide	*Primary structure*
Gal(1–29)-NH_2	Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Ile-Asp-Asn-His-Arg-Ser-Phe-His-Asp-Lys-Tyr-Gly-Leu-Ala-NH_2
M15: Gal(1–13)-SP(5–11)	Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Gal(1–14)-[Abu^8]SCY-I	Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Abu-Leu-Met-NH_2

SP, substance P.
[Abu^8]SCY-I, a molecule of scyliorhinin-I; modified by a substitution of glycine with α-aminobutyric acid.
[Abu^8] in the 8th position of the peptide chain.

Table II
A comparison of some pharmacological variables of Gal, M15 and Gal(1–14)-[Abu^8]SCY-I

Peptide	*Efficacy (%)*	*EC_{50} (nM)*	*Hill's coefficient*	*Number of experiments*
Gal	100	13.7 (6.4–29.4)	1.03	10
M15	130* (112–147)	70.1† (40.8–120)	0.73	10
Gal(1–14)-[Abu^8]SCY-I	190‡ (171–208)	187‡ (107–328)	1.56	10

Data are expressed as means with respective confidence limits. Efficacy refers to the maximum response produced by the tested peptide and is expressed as a percentage in comparison to the maximum contractile effect produced by Gal. EC_{50} were calculated from the appropriate concentration–response curves. *P<0.05; †P<0.01; ‡P<0.001.

Table III
Effects of tachyphylaxis to SP on the contractile action of Gal, M15, Gal(1–14)-[Abu^8]SCY-I

Peptide	*Concentration (nM)*	*Contractile effect (%)*	*Number of experiments*
Gal	300	95.5±2.44	9
M15	1000	42.3±3‡	11
Gal(1–14)-[Abu^8]SCY-I	3000	60.4±5†	9

The contractile response produced by a particular peptide was measured before (control) and after tachyphylaxis to SP. Results are shown as a percentage of respective controls±SEM. *P<0.05; †P<0.01; ‡P<0.001.

effects of M15 and Gal(1–14)-[Abu^8]SCY-I, by about 57.7±3% and 39.6±5%, respectively (Table III).

DISCUSSION

Tatemoto *et al.* [1] described a contractile effect of porcine Gal on rat stomach fundus strips. Our study supports previous findings suggesting that Gal contracts rat fundus by a direct interaction with a specific receptor in the smooth muscle cells [11]. Hill's coefficient for Gal is 1.03 (Table II) indicating an interaction of one Gal molecule with one receptor, fulfilling criteria of the classical receptor theory. Both analogues also seem to be contractile agents. In contrast to Gal their Hill's coefficients differ significantly from 1. For Gal(1–14)-[Abu^8]SCY-I it was 1.55, for M15 0.73 (Table II). This suggests that the principle of interaction of one drug molecule with one receptor may not apply. It could be due to activation of more than one type of receptor, negative and/or positive receptor co-operativity or multiple-step reactions of analogues with the receptor [24]. Our results seem to uphold the statements concerning M15 as a full agonist in gastrointestinal smooth muscles [14], an action dissimilar to the M15 activity in the central nervous system [12] and in pancreatic β-cells [13].

According to Katsoulis *et al.* [11] deletion of the C-terminal of Gal leads only to a minor loss of potency. We showed that substitutions of amino acids in the C-terminal part of the Gal molecule by SP or modified SCY-I cause a significant loss of potency. Conclusions that this effect may be due to the conformational changes of the Gal molecule can not be drawn at present on the basis of the experiments conducted here. Tachyphylaxis to SP decreased the effect of M15 and Gal(1–14)-[Abu^8]SCY-I on gastric smooth muscles, without any effect on the action of Gal. These findings corroborate our initial hypothesis: the action of M15 and Gal(1–14)-[Abu^8]SCY-I on smooth muscles may not only be due to their agonist activity at Gal receptors, but may result from a subsequent stimulation of receptors for SP and perhaps for other tachykinins as well. However, a possibility that Gal analogues release endogenous SP can not be excluded. Further studies involving a tachykinin antagonist spantide are in progress at the moment.

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